

# Guidelines for the monitoring of *Cerambyx cerdo*

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Academic editor: P. Audisio | Received 14 March 2017 | Accepted 29 June 2017 | Published 28 August 2017

<http://zoobank.org/DOC8D9C4-872F-4AA1-9EA7-50B413168E3B>

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**Citation:** Redolfi De Zan L, Bardiani M, Antonini G, Campanaro A, Chiari S, Mancini E, Maura M, Sabatelli S, Solano E, Zauli A, Sabbatini Peverieri G, Roversi PF (2017) Guidelines for the monitoring of *Cerambyx cerdo*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. *Nature Conservation* 20: 129–164. <https://doi.org/10.3897/natureconservation.20.12703>

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## Abstract

*Cerambyx cerdo* is a longhorn beetle widely distributed in southern and central Europe. This saproxylic beetle is generally associated with oak forests where there are mature or partially dead and sun-exposed trees. Its populations are currently threatened by forest practices such as the removal of partially dead trees and the decline in the number of old oak trees situated in open or semi-open landscapes. Thus, *C. cerdo* has been included in Annexes II and IV of the Habitats Directive. The present paper is part of a special issue on monitoring of saproxylic beetles which are protected in Europe, based on the research carried out during the LIFE-MIPP project, with a revision of the current knowledge on systematics, ecology and conservation of *C. cerdo*. The main aim of the present paper is to test different monitoring methods in order to develop a quick and reproducible protocol for the conservation of this species. The methods tested were: artificial sap attracting the adults, baited traps, VES (visual encounter survey) and collecting remains of predation along transects. Based on these results, a detailed monitoring method for *C. cerdo* using baited trap is proposed in this paper, together with a discussion on its constraints, spatial validity and possible interferences. In order to assess the conservation status of populations of *C. cerdo* in Europe and to compare populations over time, a method for the calculation of a reference value, based on the monitoring method, is provided.

**Keywords**

Habitats Directive, Saproxylic beetles, Coleoptera, Cerambycidae, Monitoring methods, Forest biodiversity, Baited trap

**Introduction**

The great capricorn beetle, *Cerambyx cerdo* Linnaeus, 1758, is a large longhorn beetle (Coleoptera: Cerambycidae), generally associated with oak forests where there are mature or partially dead and sun-exposed trees. It is listed in Annexes II and IV of the Habitats Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora). The Habitats Directive provides that Member States of Europe prepare, every six years, a report on the conservation status of the threatened species listed in the Annexes. In order to address this obligation, the Life Project “Monitoring of insects with public participation” (LIFE11 NAT/IT/000252) (hereafter, MIPP) conducted experimental fieldwork to develop standardised methods for monitoring of the saproxylic beetle species of the project: *Osmoderma eremita* (hermit beetle, Scarabaeidae), *Lucanus cervus* (European stag beetle, Lucanidae), *Rosalia alpina* (rosalia longicorn, Cerambycidae), *Morimus asper funereus* (morimus longicorn, Cerambycidae) and *Cerambyx cerdo* (great capricorn beetle, Cerambycidae).

The present paper is part of a special issue on monitoring the abundance of saproxylic beetles protected in Europe and is dedicated to *C. cerdo*. Therefore, it starts with an extensive revision of the current knowledge on systematics, distribution, ecology, ethology and conservation of this species. The review is followed by a detailed account of the fieldwork carried out during the MIPP project and concludes with a description of the proposed monitoring method.

**Systematics and distribution**

The genus *Cerambyx* includes 13 species in the Palaearctic region, at least 7 species of which occur in Europe (Müller 1950, Švácha and Danilevsky 1987, Bense 1995, Özdi̇kmen and Turgut 2009).

*Cerambyx cerdo* (Linnaeus, 1758), *C. scopolii* Fuesslins, 1775, *C. miles* Bonelli, 1823 and *C. welensii* (Küster, 1846) are more or less widely distributed in Europe, with the last two taxa mainly restricted to the southernmost countries, whereas *C. nodulosus* Germar, 1817, *C. dux* (Faldermann, 1837) and *C. carinatus* Kuster, 1846 occur only in eastern European countries, especially in the Balkan-Mediterranean habitats (Bense 1995).

*C. cerdo* occurs in Europe, Caucasus and in the Middle East up to northern Iran. This species is widespread in most parts of Europe (northwards to southern Sweden and Great Britain eastward to Belorussia, Moldavia, Ukraine and Crimea) but is more

common in the Mediterranean region (Sama 1988). In Italy, it is widely distributed, occurring also in the Tyrrhenian islands but being absent in the northwestern part (the Aosta Valley) (Sama 1988, 2002). *C. cerdo* is known to live sympatrically with *C. welensii* and to have an ecological niche similar to the latter, as in Southern Spain (Torres-Vila et al. 2012, 2013) and in the MIPP study area of Bosco della Fontana, in the Po valley. *C. welensii* (often still quoted under the synonym *C. velutinus* Alfieri, 1916) is widespread in southern Europe, reaching eastwards to Ukraine and Iran.

Different subspecies are described under the taxon *C. cerdo*: *C. cerdo pfisteri* (Stierl, 1864), *C. c. acuminatus* Motsch, 1852, *C. c. mirbecki* Lucas, 1849 and *C. c. iranicus* Heyrovský, 1951 (Müller 1950, Villiers 1978, Sama 1988, Özdi̇kmen and Turgut 2009). However, the validity of most of these subspecies has been cast in doubt by Sama (2002).

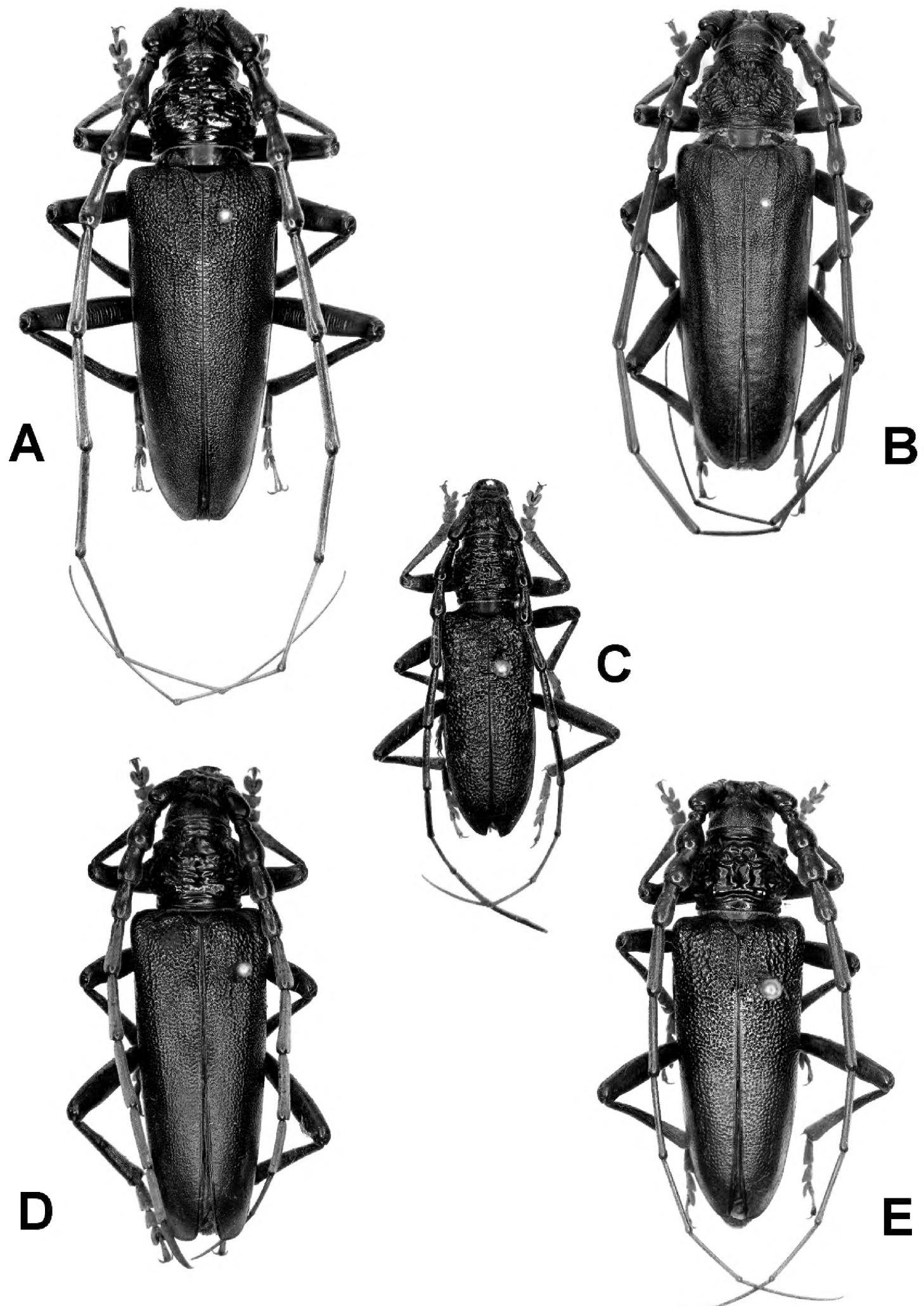
## Morphology

The adult specimens of *C. cerdo* are 17–56 mm long (excluding the antennae) and 8–14 mm wide, with a body overall blackish and elytra reddish-brown towards the distal portions. The head is provided with strong mandibles and is transversally rugose on the upper side (vertex). The antennae are long, as in most species of the Cerambycidae family: in females, the antennae are long like the main body length (last antennal segments reaching at least the distal part of the elytra), while in males, the antennae are much longer than the body (the last 3 or 4 segments of the antennae exceed the distal margin of the elytra). In males, the last segment of the antennae is much longer than the previous one, while in females, the last segment is as long as the previous one or shorter. The pronotum is heavy sculptured and shows a conspicuous thorn laterally on both sides. Elytra are rugose, densely punctate, with rugosity decreased in the distal part and are truncated at their apex (Rudnew 1936, Villiers 1978, Bense 1995, Harde 1996, Özdi̇kmen and Turgut 2009).

The larvae of *C. cerdo* look like those of many other longhorn beetles, with generally creamy-white-yellowing body and reduced legs. The full grown larvae are up to 70–90 mm long, 18–20 mm broad; head white-yellow with widely pigmented and strongly sclerotised black-pitchy-brown mouth frame and black mandibles. Pronotum is provided with sclerotised shield; legs are very short but distinct (Rudnew 1936, Villiers 1978, Švácha and Danilevsky 1987).

## Identification and comparison with similar taxa

Five species belonging to the genus *Cerambyx* often occur together in forest ecosystems of Italy and other south-central or western European countries: *C. cerdo*, *C. miles*, *C. scopolii* and *C. welensii* (Figure 1). *C. nodulosus* (an eastern species related to *C. miles*) occurs only in Friuli Venezia Giulia (Sama and Rapuzzi 2011).



**Figure 1.** The most widespread species of *Cerambyx* in Europe: **A** *C. cerdo* **B** *C. welensii* **C** *C. scopolii* **D** *C. miles* **E** *C. nodulosus* (photo by Pierpaolo Rapuzzi).

*C. welensii* and *C. cerdo* can be distinguished from *C. miles* and *C. scopolii* by the shape of the inner elytral apex which bears a small acuminate tooth. Furthermore, *C. scopolii* is small (17–28 mm) and entirely black, often found on flowers of elders (*Sambucus*) and other shrubs. The elytra of *C. cerdo* have the anterior portion deeply sculptured, black, shiny, almost glabrous, tendentially restricted and subtruncated at apex; those of *C. welensii* are evenly brownish, weakly sculptured, covered with minute setae, sub-parallel and rounded at apex. *C. cerdo* and *C. miles* have the elytra black and shiny, deeply sculptured and with red apex; however, the latter does not have the terminal elytral tooth and shows the first four or five antennal segments short and thick. In males of *C. welensii*, antennae exceed the body length with the last three antennal segments (never with the last four as in some *C. cerdo* males). Females of these two species are distinguishable by the length of antennae, extended to the apical third of elytra in *C. cerdo* and only to the middle in *C. welensii* (Pesarini and Sabbadini 1994). In eastern European countries, the presence of additional species, whose identification is often hard and the taxonomic status questioned, makes the fieldwork more problematic for non-expert operators. The detailed and updated distribution of the species in Europe can be found in the TITAN Cerambycidae database (Tavakilian and Chevillotte 2017).

## Ecology

*C. cerdo* is a polyphagus saproxylic species that usually lives in deadwood of standing living veteran oak trees (*Quercus* spp.) and other deciduous species such as *Castanea sativa*, *Juglans regia*, *Fraxinus* spp., *Salix* spp., *Ulmus* spp., *Fagus sylvatica*, *Platanus* spp., *Prunus* spp. (Müller 1950). *C. cerdo* generally occurs in semi-open wood stands which also match the habitat requirements of other saproxylic species like *Osmoderma eremita* Scopoli, 1763 (Coleoptera Scarabaeidae) and some other highly endangered beetles (Ranius and Nilsson 1997, Buse et al. 2007).

Habitats of *C. cerdo* are lowland and hilly forests comprising various species of *Quercus* (Sama 1988). Viable populations of *C. cerdo* can also be found in tree avenues or parks (Buse et al. 2007, Carpaneto et al. 2015). Colonised trees can be identified by visible holes made by the larvae on the trunk or thick branches (Müller 2001). These holes can persist over many years or even decades; typical signs of recent activity are wood meal and fresh holes with red-coloured interior sides (Buse et al. 2007). Studies on *C. cerdo* habitat preferences highlighted that the bark depth of the trees is one of the most significant predictors for species presence and increasing age and diameter of the oak trees improve the probability of occurrence. The vitality of the trunk, the insolation of the tree and the habitat openness appeared to be other important parameters for predicting the presence of this saproxylic beetle (Buse et al. 2007). Factors affecting the distribution of *C. cerdo* amongst individual trees have been studied by Albert et al. (2012). The authors demonstrated how the presence of exit holes in the trunks of large old oaks was positively associated with the diameter of the trunk and insolation of the

tree, while the number of exit holes decreased with the height above the ground. When a tree is attacked by *C. cerdo*, the tree may survive over long periods, although its survival will be determined by the increasing amount of dead wood on it. Furthermore, the larvae create extensive galleries (Palm 1959), which may act as entrances and habitats for other species. Buse et al. (2008a, 2008b) compared the saproxylic assemblage of colonised and uncolonised trees in order to study the functional role of *C. cerdo* as an ecosystem 'engineer' by using the interceptor traps. Results suggested that *C. cerdo* can alter its own habitat to create favourable habitat conditions for entire assemblages of saproxylic insects.

## Larval ecology

The larval development of *C. cerdo* mainly takes place in fresh wood of oaks (*Quercus* spp.) and lasts about 3–4 years, producing an irregular pattern of larval galleries (Pavlović et al. 2012). During the first year, the larvae feed under the bark layer, while in the second year, they move deeper into the wood (Neumann 1985, Bense 1995). Larval development was observed not only in tree trunks but also inside large branches (Campanaro et al. 2011a). Larvae, after completing their development, excavate a last tract of gallery directed toward the surface of the trunk and then back into the tree, where they are protected by any contact with the external environment; here, pupation occurs generally during May or June. The adult remains inside the pupation chamber or close to the exit hole until the following spring. During spring and summer, adults emerge from colonised trees by producing large and ellipsoidal exit holes into the bark (Horák et al. 2010). Exit holes are easily detectable but are not species-specific and can be confused with holes dug by other *Cerambyx* species. The wood is perforated by deep, broad and winding galleries and emergence holes are characterised by their large size and oval shape (length 3 cm, width 1.5 cm on average) (Luce 1997). In central Europe, the larvae usually develop in *Q. robur* or *Q. petraea*, but in southern countries, they have also been found on *Q. pubescens*, *Q. ilex*, *Q. suber* and *Castanea sativa* (Luce 1997). Although suitable trees have a sun-exposed trunk with at least 60 cm in diameter (Buse et al. 2007), in central Italy, smaller trees suitable for *C. cerdo* have been reported by Marianelli et al. (2011).

## Adult ecology

The adults remain sheltered in their chambers during the winter (Horák et al. 2010) and emerge between May and August, depending on local climatic conditions relating to altitude and latitude. In central and southern France, where the species is more widespread, the adults are active for a longer period, from June to September (Bensetti and Gaudillat 2002). In Lower Saxony (Germany), the emergence of the adult great capricorn beetle generally occurs from the end of May until the beginning of August,

although later observations have been reported throughout August and September (Buse et al. 2007). In south-western Hungary, the adults are active by mid-spring (early May), remaining active into mid-summer (Keszthelyi 2015). In the Iberian Peninsula (in the northern part of the province of Alicante), they are active during spring and summer, particularly from early May to late August. In some cases however, adult activity spans from February to June, due to higher average temperatures in south-eastern Spain (Peris-Felipo and Jiménez-Peydró 2011).

Old oaks are the preferred habitat of *C. cerdo*. In France, the beetle colonises oaks and chestnut trees below the altitude of 900 m a.s.l. (Horák et al. 2010). In Italy, the great capricorn develops mainly in various species of *Quercus* and has also been reported in other hardwood trees (Sama 1988). In Germany, the species developed exclusively in oak species: *Q. robur* is the preferred development tree, followed by *Q. petrea* (Neumann and Schmidt 2001) and *Q. rubra* (Nessing 1988). According to Neumann and Schmidt (2001), emergence holes belonging to *C. cerdo* have also been recorded on *Q. coccinea*, a non-native oak species introduced from North America in 1691. In Hungary, this species lives mainly in turkey oak woodlands on hill and lower mountain areas. Important populations occur in wood pastures and parklands where huge, old, solitary oaks occur. In Romania, it has been reported from all of oak species present in the country. In the European part of Turkey, larvae of *C. cerdo* developed in trees species belonging to the genus *Acer*, *Carpinus*, *Castanea*, *Cupressus*, *Fraxinus*, *Fagus*, *Platanus*, *Prunus*, *Salix*, *Quercus* and *Ulmus* (Horák et al. 2010). Adults of *C. cerdo* are mainly nocturnal and have a lifetime of a few days up to two months and they feed on mature fruit and oak sap (Weckwerth 1954, Döhring 1955, Neumann 1985).

The dispersal biology of the species is poorly known, the adults flying mainly after dusk when the temperature exceeds 18°C (Neumann 1985), but sometimes they have also been spotted in the afternoon (Müller 1950, Campanaro et al. 2011a).

In literature, no information has been found on predators of adults. It is however very likely that some mammal and bird species regularly prey on them.

## Life cycle

Mating takes place during summer, when females lay their eggs in tree bark crevices or damaged parts of previously colonised oaks. Laboratory tests demonstrate that the maximum daily fecundity ranged widely, depending on the egg-laying day and female size from about 5 to 15–20 eggs/day, with some large females laying up to 30 eggs in a single day (Torres-Vila 2017). In the same study, Torres-Vila (2017) reports the measurements of the eggs of *C. cerdo*, (length × width: 3.7 × 1.9 mm<sup>2</sup>) with mean dimensions slightly longer than those previously reported by other authors: 3.3 × 1.6 mm<sup>2</sup> (Marović 1973), 2.5–3 × 1 mm<sup>2</sup> (El Antry 1999) and 3.1 × 1.5 mm<sup>2</sup> (Vitali 2001). Larvae hatched from eggs have a more phloem-feeding habitus in the first year of life (they feed in the cortical part of the trunk) and then, in the following year, they start to dig tunnels that penetrate deeper into the xylem, altering sap flow and triggering

leaf fall and tree decay (Neumann 1985, Bense 1995). According to Marović (1973), it is possible to rear *C. cerdo* larvae in the laboratory where the larval development can be approximately one third shorter than in the wild (Nenadović et al. 1999, Pavlović et al. 2012). The transformation from larva to adult takes place inside the pupal cell near to the surface of the trunk. The pupae are large and light coloured initially, gradually darkening until the appearance of the adult in the autumn, usually in October.

## Threats and conservation

Over the last century, European populations of *C. cerdo* have suffered a dramatic decline in the number of populations and in population sizes in the whole of central Europe (Klausnitzer et al. 2003). This is mainly due to a decline in the number of old oak trees situated in open or semi-open landscapes, this representing the main threat for this species (Luce 1997, Dupont and Zagatti 2005, Buse et al. 2008a); in the Great Britain and Swedish mainland, the species is today considered extinct (Alexander 2002, Ehnström and Axelsson 2002). *C. cerdo* is classified as Vulnerable (VU), globally Vulnerable, according to the IUCN Red List of Threatened Species (World Conservation Monitoring Centre 1996). This species is listed in: (i) Appendix II of the Bern Convention as a strictly protected species, (ii) Annex II of the European Habitats Directive as a non-priority species and (iii) Annex IV of the European Habitats Directive.

## Review of monitoring experience in European countries

Although *C. cerdo* is considered a threatened species in most parts of its range, a long-term monitoring programme has never been conducted. As reported by Campanaro et al. (2011a), several European countries highlighted the necessity to develop monitoring methods for this species in order to obtain information about its populations' consistency, demographic trends and habitat range, as required by the Habitats Directive (92/43/EEC). Actually, until now, no long-term monitoring projects have been carried out, except in Slovenia (Vrezec et al. 2007) and in Germany (Schnitter et al. 2006). On the other hand, some protocols for monitoring have been proposed and tested. These methods are briefly discussed below.

### ***Capture-Mark-Recapture (CMR)***

Campanaro et al. (2011a) proposed preliminary guidelines for the conservation and monitoring of *C. cerdo* and other saproxylic species in Italy by using the capture-recapture protocol. Briefly, adults were trapped with air traps baited with a mixture of vinegar (or wine or beer) and fruit (e.g. banana) during the flight period (May-August). The traps were positioned on trees where recent exit holes of beetles were found.

Beetles were marked with individual codes (colours or/and numbers) on the ventral side of the specimen (thoracic sternites or/and abdomen) using a permanent, non-toxic and odourless fine-tip marker. This method allows the estimation of the size of the population, but requires a significant sampling effort and a large number of person-hours for repeated sessions of capture and recapture. On the basis of the marked and recaptured individual ratio, it is possible to estimate the number of individuals in the population (Amstrup et al. 2005, Hill et al. 2005, Campanaro et al. 2011a, Trizzino et al. 2013). Capture-recapture estimates were also carried out on the closely related species *C. welensii* by López-Pantoja et al. (2008) and Torres-Vila et al. (2012, 2013) in Spain. The capture-recapture protocol using feeding traps (red wine, vinegar and sugar), integrated with nocturnal observation, was undertaken by Torres-Vila et al. (2012, 2013), while nocturnal observation was the method used by López-Pantoja et al. (2008).

### ***Surveying the exit holes***

In literature, there are essentially two kinds of contributions that focus on exit holes;

- (i) the paper of Albert et al. (2012), who investigated the pattern of distribution of *C. cerdo* within the old open-grown oaks at two sites in Bohemia (Czech Republic). The colonised trees were identified by exit holes (Buse et al. 2007) for a total of 30 oaks with 4,279 holes. Each tree was climbed and the number of exit holes and environmental variables were recorded in relation to trunk sections (height 2 metres) and segments (orientation of the trunk). This study revealed that *C. cerdo* prefers trunks of large and old trees in open areas, especially in the sun-exposed lower parts of the trunks. “Searching the exit holes method” facilitates the detection of the presence of the species in an easy manner and it can be proposed as an effective method, although the sampling effort in climbing trees is significant.
- (ii) the works of Buse et al. (2007, 2008a and 2008b). Buse et al. (2007) studied the distribution modelling pattern of the populations of *C. cerdo* in Germany in order to understand the species–habitat relationships and its environmental requirements. The presence data of the species were obtained by searching the exit holes on trees in two sites in Lower Saxony. They investigated more than 250 trees and identified that 67 of these had recently been colonised by *C. cerdo*. The results of this habitat suitability model showed that oak tree-level parameters, such as trunk insolation, presence of sap, bark depth and the distance from the next colonised tree, were able to predict the presence of *C. cerdo* much better than landscape-level predictors. It was concluded that, to facilitate the survival of the populations of *C. cerdo* and other similar saproxylic species, the management strategies should focus on semi-open woodstands (Ranius and Nilsson 1997, Buse et al. 2007).

In Germany, a standard monitoring approach based on the survey of the exit hole has been performed every five years since 2006. The field activity to estimate the popu-

lation size is performed before the flight period of the adult, from September to April of the following year, by counting the number of exit hole on selected trees (e.g. n=10) per area. The number of selected trees depends on the number of colonised trees; in case the number of suitable trees is less than six, all trees should be considered (Schnitter et al. 2006).

## Methods

### The methods used during MIPP

During MIPP, several methods were tested for monitoring *C. cerdo*: (i) Artificial sap, (ii) Baited traps, (iii) Collecting remains of predation along transects and (iv) Visual Encounter Surveys (VES). These methods are discussed below:

#### *Artificial sap*

Manna is the sap extracted from the bark of several ash tree species (*Fraxinus*), particularly from *F. ornus* (manna ash). Many saproxylic beetles at the adult stage (including several longicorns), feed on mature fruit and on sap that flows out from the bark of trees (Neumann 1985). Observations showing that *C. cerdo* is attracted by oak sap on trees were reported by Weckwerth (1954) and Döhring (1955). Exploiting the characteristic smell of fermentation, as in baited traps, the method was aimed at mimicking the chemical component of oak sap and testing the attraction of the manna. Jansson (2011), for a similar study on *Lucanus cervus* (Linnaeus, 1758), used artificial sap prepared from pieces of oak bark and equipment for homemade wine. It was decided to use manna obtained from *Fraxinus* due to its commercial availability. As manna is generally sold in solid form as pieces of medium size, it was reduced to small pieces with a mixer and afterwards, water was added to obtain a final solution with a creamy consistency. The right consistency of the solution was obtained by mixing 150 g of manna reduced to small pieces with 60 cm<sup>3</sup> of water. This product was smeared with a brush on to the tree bark of selected trees to produce some 'feeding stations'. Each feeding station corresponded to a bark surface of 5 × 15 cm of smeared solution. The solution was added to bark in the afternoon and during the night between 21:30h and 22:30h, when the surveys were undertaken. Trees were selected for the presence of emergence holes of *Cerambyx* spp. (evidence of breeding) and for the absence of holes (as control) (Figure 2).

#### *Baited traps*

The baited traps used for the present research were the same as built by Campanaro et al. (2011a) and Bardiani et al. (2017a, 2017b) for monitoring *Cerambyx cerdo* and



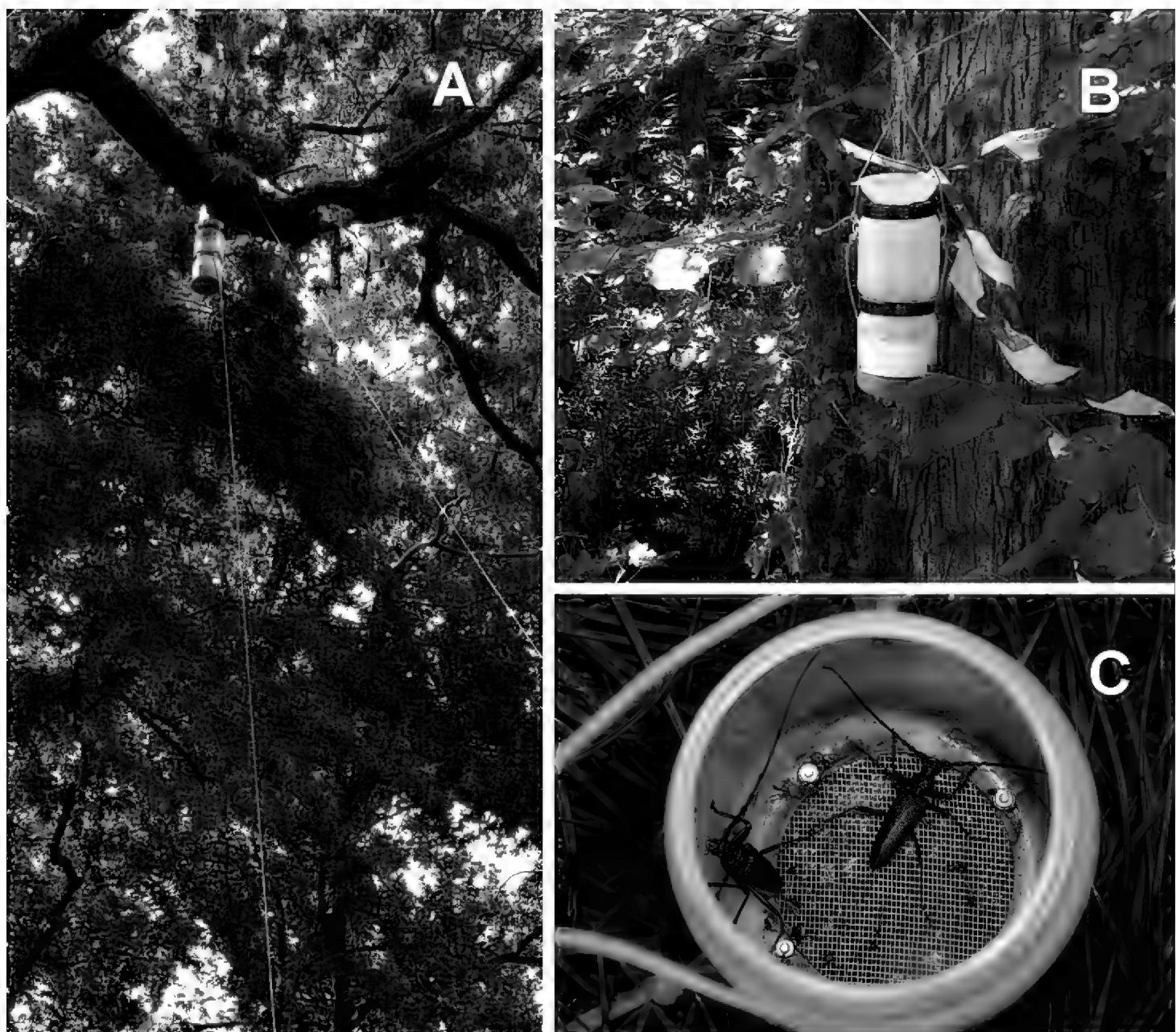
**Figure 2.** Tree with manna solution smeared on the bark, used as feeding station for longhorn beetles (Photo by L. Redolfi De Zan).

*Lucanus cervus* respectively. They were obtained by modifying a previous aerial and baited trap model invented by Allemand and Aberlenc (1991) for trapping saproxylic beetles and chosen by several other authors for monitoring *C. cerdo* (Mason et al. 2002,



**Figure 3.** The upper jar of the baited trap modified with a wire insect net and a modified lid in which a plastic funnel has been inserted to collect insects falling into the trap (Photo by M. Bardiani).

Vrezec and Kapla 2007), *C. welensii* (Torres-Vila et al. 2012) and other beetles. The method was based on the attractive power of a bait (e.g. a food source that simulates a fermented sap, such as red wine + beer + sugar or squashed banana) poured into a plastic cylindrical container which was closed by a funnel (Allemand and Aberlenc 1991). Nevertheless, for the purpose of monitoring this protected species, these traps were modified (Campanaro et al. 2011a, Corezzola et al. 2012) to be less invasive and to prevent the death of the animal in the attractive liquid. Traps were all handcrafted and composed of two stacked HDPE plastic jars (1000 cm<sup>3</sup> each) fitted together one above the other, with a wire insect net (2 × 2 mm mesh) between them (in the upper bottle, the bottom was previously drilled with a hole of 76 mm diameter) (Figure 3). The lower jar contained the bait as a liquid mixture and the upper one represented the capture chamber. The wire net ensured that individuals survived in the trap by avoiding any contact with the liquid of the bait. The upper jar had the lid modified with a plastic funnel (diameter: 10 cm; with the stem cut out to create an opening of 4 cm diameter). Two mixtures were tested as baits: (i) red wine, beer and mashed banana ( $R_w B_e B_a$ ) previously tested by several authors (Allemand and Aberlenc 1991, Campanaro et al. 2011a) and (ii) red wine, white wine and sugar ( $R_w W_w S$ ) (suggested by P. Rapuzzi, a long horn beetles taxonomist). The mixtures were prepared a week before the setting of traps. To each litre of mixture made up by 50% of each of the two liquids



**Figure 4.** Baited traps set at two heights: **A** on a branch over 10 m high **B** on trunk, 1.5–2 m above understory level and **C** an example of capture of two individuals of *Cerambyx cerdo*. In picture **A** the ropes to lower and lift up the trap are visible, green and brownish respectively (Photo by M. Bardiani).

(red wine + beer or red wine + white wine), 220–330 g of banana or 220 g of sugar were added. The final volume of the bait per trap was about 500 cm<sup>3</sup>. Traps were set at two heights: on the trunk at about 1.5–2 m high, to investigate the underground layer and on branches over 10 m high to investigate the lower canopy (the compact design and low weight of the traps allowed an easy lift up between the branches) (Figure 4). For canopy trap setting, a tree-climb slingshot (BigShot by Sherrill tree) was used for the launch of a rope, to which the trap was then tied. This rope allowed the trap to be lifted. Another rope was tied to the bottom of the trap to lower it. Trap checking was performed daily (to avoid any cases of death of collected individuals), in the morning (from 08:00h to 13:00h). Traps were activated on Monday, remained active for 4 days (i.e. surveys) and deactivated on Friday. No interception devices (e.g. panels in windows traps) were mounted on the traps, as the aim of this test was to evaluate only bait attraction efficacy and to reduce passive capture of both *C. cerdo* and other species.

### Collecting remains of predation along transects

This method was based on the search and collection of remains of *C. cerdo*, in a similar manner to several monitoring and sampling studies on *L. cervus* (Campanaro et al. 2011b, Harvey et al. 2011, Bardiani et al. 2017a). The collection of remains was undertaken along transects of standard length (500 m long) and described in detail by Bardiani et al. (2017a). The collection of remains was carried out by one surveyor during daytime. All remains were collected and preserved dry in paper bags. The date, identification code of the bag, transect and sector of collection, number of specimens (counting is performed considering all the body parts reasonably belonging together as one specimen) and type of remains collected (e.g. elytra, pronotum, head, whole specimens) were recorded for each bag.

### VES

This method was based on detecting active adults of *C. cerdo* on trunks (Figure 5). For this reason, a headlight was necessary (Tikka XP2, Petzl) or a torch (Led Lenser M7R) to light up trunks and lower parts of the canopy respectively. Binoculars were used to check higher parts of the tree. Sightings were divided according to the height of obser-

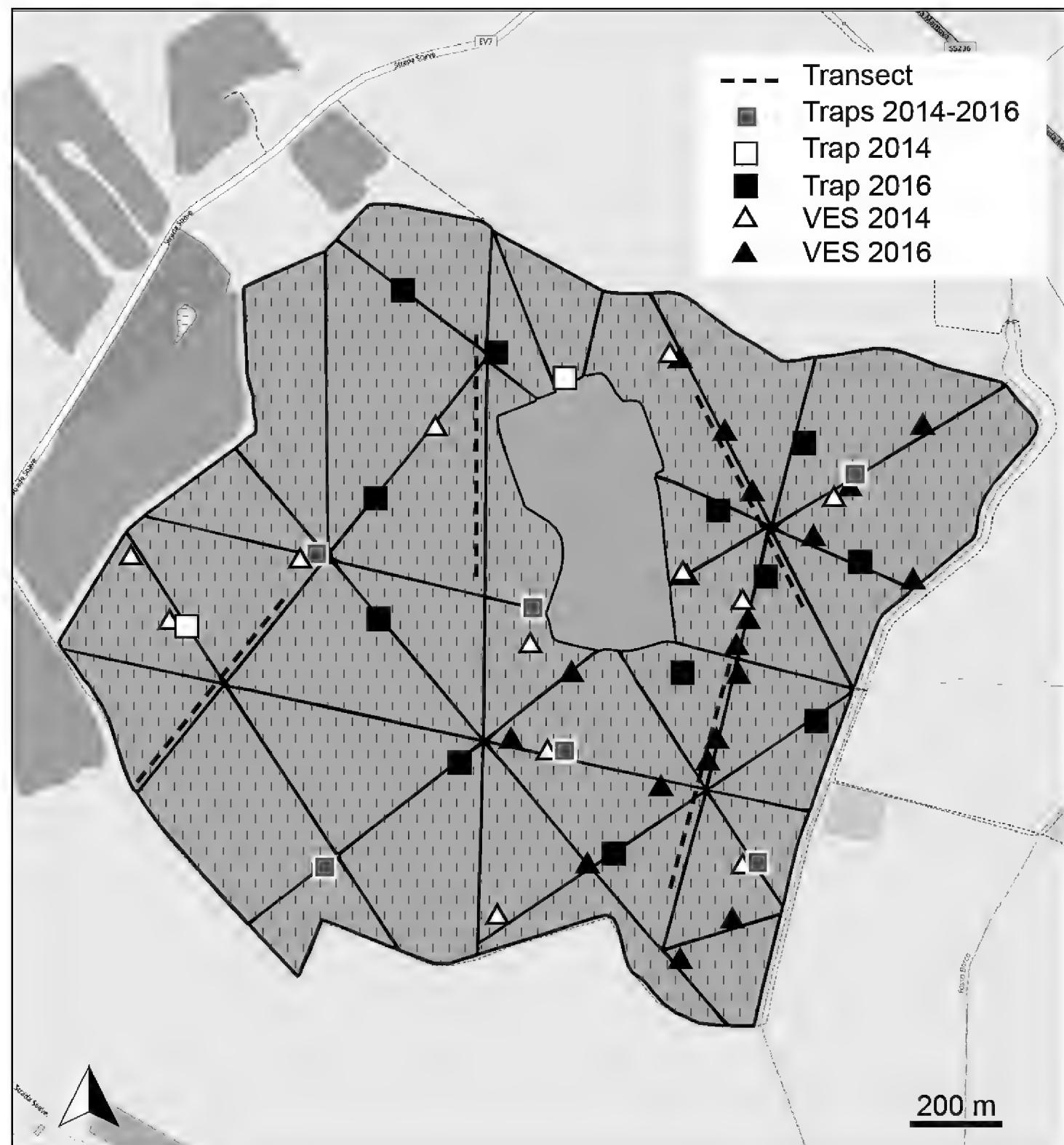


**Figure 5.** An individual of *Cerambyx cerdo* observed on tree selected for the VES (Visual Encounter Surveys) (Photo by S.G. Muñoz).

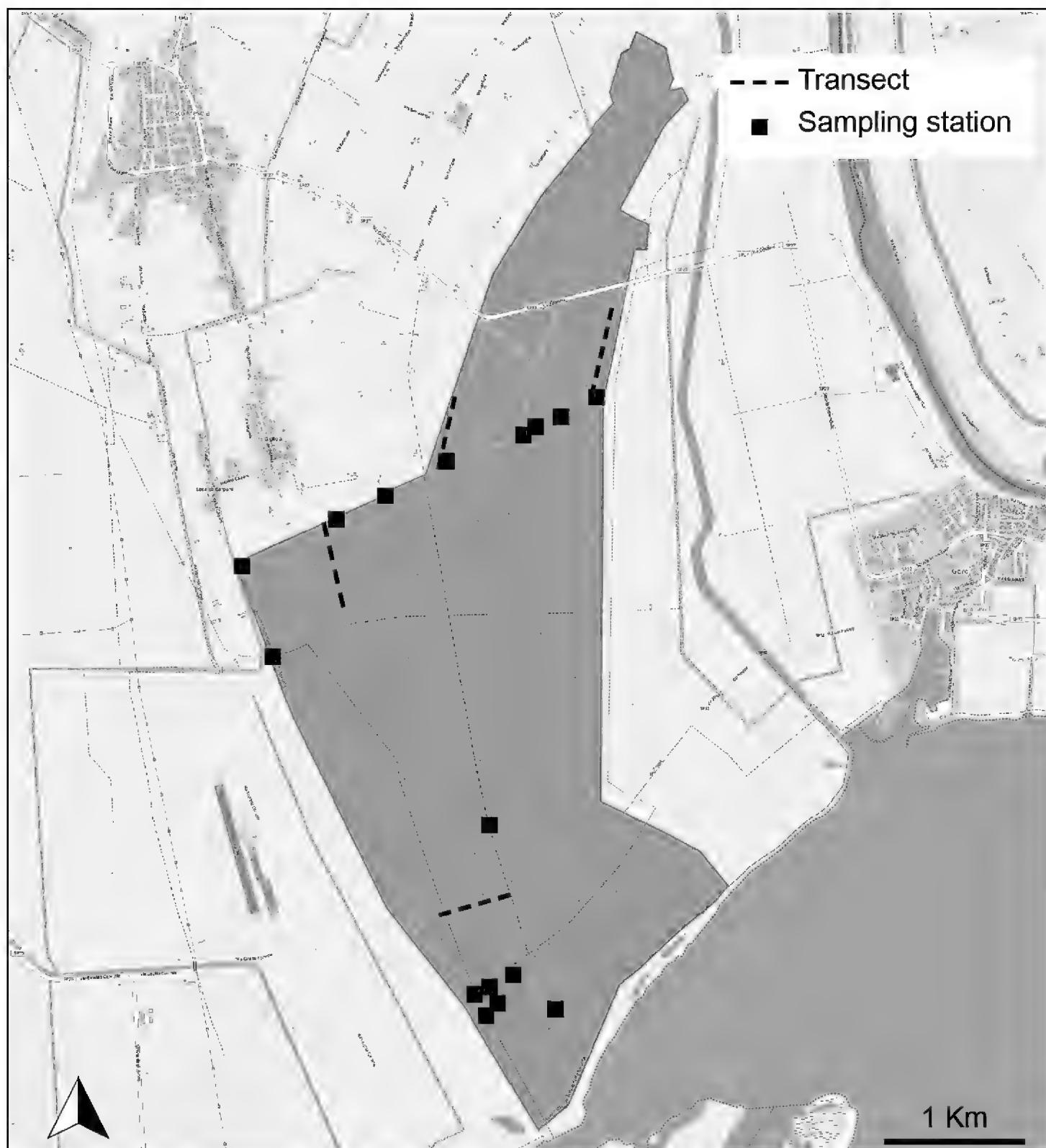
vation: up to 2 m and over 2 m. In case of co-occurrence of more *Cerambyx* species, individuals not identified were indicated as *Cerambyx* sp.

## Sampling plan

The methods explained above, were tested in two study areas: Bosco della Fontana and Bosco della Mesola (Figures 6 and 7), both in the Po valley, during 2014, 2015 and 2016 (see Carpaneto et al. 2017 in this issue, for the description of the study areas).



**Figure 6.** Map of the study area Bosco della Fontana.



**Figure 7.** Map of the study area Bosco della Mesola.

The method “Artificial Sap” was tested only in 2015 at Bosco della Mesola, from 25 May to 17 July (Table 1). Two kinds of feeding station were used: (i) on trunks of a potentially suitable tree for the presence of *C. cerdo* and (ii) on trunks of a tree without signs of *C. cerdo*. All trees selected were oaks (*Quercus ilex* and *Q. robur*) and suitability was established on the base of vitality and openness of the canopy, presence of exit holes, presence of sap and bark status (Buse et al. 2007). The feeding stations were distributed in 16 replicates: each replicate had both types of feeding stations (overall 32 oaks were selected). Each replicate was checked once a week (eight replicates on Tuesday and eight on Thursday) from 21:30h to 22:30h. In the afternoon (about 18:00h–19:00h), the manna of each feeding station was moistened by spraying water on it.

**Table 1.** Sampling plan at Bosco della Fontana (BF) and Bosco della Mesola (BM) during the three years of monitoring. N = number of transects or traps; S = number of surveys; \* indicates the number of surveys for each transect.

Site	Method	2014			2015			2016		
		N	S	Dates	N	S	Dates	N	S	Dates
BF	Baited traps	48	32	27.5–18.7	-	-	-	54	24	31.5–8.7
	Remains	-	-	-	4	10*	20.5–22.7	4	7*	1.6–14.7
	VES	16	7	3.6–16.7	-	-	-	20	6	31.5–7.7
BM	Artificial sap	-	-	-	32	8	25.5–17.7	-	-	-
	Baited traps	-	-	-	32	32	25.5–17.7	-	-	-
	Remains	-	-	-	4	8*	25.5–17.7	-	-	-
	VES	-	-	-	16	8	25.5–17.7	-	-	-

The method “Baited traps” was tested in different numbers and in different years in the two study areas (Tables 1 and 2). During 2014, at Bosco della Fontana, the degree of attraction of the baits and height positions of the traps were tested. Overall 48 traps were set, arranged in eight replicates. Each replicate consisted of six traps, set in pairs on three oaks (*Q. cerris* and/or *Q. robur*) with a diameter at breast height (DBH) greater than 50 cm measured at 130 cm from the ground: one trap was set at canopy level, between 10 m and 20 m high and another at ground level on the lower part of the trunk (1.5–2 m height). For each replicate, two baits were tested: the  $R_w B_e B_a$  in the first pair of traps and the  $R_w W_w S$  in the second pair (the traps of the third pair were left empty and used as control). Traps were checked for eight weeks from 27 May to 18 July (Table 1). At the beginning of each week, the pairs of traps were moved between the trees selected to change position within the replicate. In 2015, traps were set only at Bosco della Mesola: the overall number of traps was 32, on 16 trees (*Q. ilex* and/or *Q. robur*), with the same trap-setting for each tree selected. Traps were checked for eight weeks, from 25 May to 17 July (Table 1). The  $R_w W_w S$  was the only bait used (Table 2). For each trap, at the beginning of the 5<sup>th</sup> week, the bait was replaced by a fresh one. In 2016, traps were tested at Bosco della Fontana. Overall, 54 traps were set over 10 m high (no traps were set at ground level) and  $R_w W_w S$  was the only bait tested. Each trap was set on its own tree (*Q. cerris* and/or *Q. robur*, with a DBH of at least 50 cm). Traps were arranged in 18 trap replicates (3 traps per replicate) and, for each one, bait was tested at 3 different ageing stages (one per trap): (i) the mixture was never changed during the study season ( $A_{NC}$ ), (ii) the mixture was replaced every three weeks ( $B_{3W_s}$ ) and (iii) the mixture was replaced every two weeks ( $C_{2W_s}$ ) (Table 2). Traps were checked for 6 weeks to test bait ageing. Testing for two extra weeks was undertaken after the 6<sup>th</sup> week, to investigate the last part of the season. Overall 32 surveys were undertaken from 31 May to 22 July. During the season, the position of the baits within the replicates never changed.

The method “Collecting of remains of predation along transect” was undertaken in 2015 in both study areas, whereas only at Bosco della Fontana in 2016. For each study area, four transects were selected. For both years, at Bosco della Fontana, the

**Table 2.** Overview of the trap sample and number of traps set in the two study areas, Bosco della Fontana (BF) and Bosco della Mesola (BM), during the three years of monitoring. Baits:  $R_w B_e B_a$  (Red Wine, Beer, Banana);  $R_w W_w S$  (Red Wine, White Wine, Sugar); Control (empty traps used as control). Height at which traps were set: High (above 10 m), Low (1.5–2.0 m). Variation in ageing of the mixture: Never (mixture never changed during the season); 3 weeks and 4 weeks (mixture changed once during the season: after three or four weeks); 2 weeks (mixture changed twice during the season: every 2 weeks).

Bait	Height	Ageing	BF		BM 2015
			2014	2016	
$R_w B_e B_a$	High	Never	8	-	-
	Low	Never	8	-	-
$R_w W_w S$	High	Never	8	18	-
		4 weeks	-	-	16
		3 weeks	-	18	-
		2 weeks	-	18	-
	Low	Never	8	-	-
		4 weeks	-	-	16
		3 weeks	-	-	-
		2 weeks	-	-	-
Control	High	-	8	-	-
	Low	-	8	-	-

transects were the same used by Bardiani et al. (2017a) for the monitoring of *L. cervus*. Each transect was monitored once a week and the survey undertaken in the same day.

The method “VES” was undertaken in all three years (Table 1): 2014 and 2016 at Bosco della Fontana; 2015 at Bosco della Mesola. In the first year, 2014, 16 oaks (*Q. cerris* and/or *Q. robur*) were chosen: eight replicates close to the baited traps and eight in the rest of the Reserve. Following Buse et al. (2007), oaks were potentially suitable for colonisation by *C. cerdo*, selected on the basis of at least one of the following parameters: vitality and openness of the canopy, presence of exit holes, presence of sap, and bark status. For all trees, DBH was higher than 50 cm. Each tree was checked in four time-slots (09:00h–11:00h; 14:00h–16:00h; 19:00h–21:00h; 23:00h–01:00h), once a week (usually carried out in two slots on Tuesday and two slots on Thursday) from 3 June to 16 July (Table 1). Sixteen oaks (*Q. ilex* and/or *Q. robur*) were chosen in 2015 at Bosco della Mesola, following the same parameters for 2014 (with the exception of the value of tree diameters, as in very few trees, it was higher than 50 cm). Each tree was checked from 25 May to 17 July (Table 1), once a week (eight trees on Tuesday and the other eight on Thursday) but only in one single time-slot (21:30h–22:30h). Finally, in 2016, 20 oaks were selected following the same parameters of 2014. Each tree was checked in one single time-slot (21:30h–22:30h), once a week (10 trees on Tuesday and 10 on Thursday) from 31 May to 7 July (Table 1).

For all methods, suitability parameters of each tree were recorded and reported on field sheets (See Suppl. material 1: Field sheet 1).

## Data analysis

Occupancy models were applied on captures data obtained at Bosco della Fontana only using the method “Baited Traps”; the methods “VES” and “Collecting of remains of predation along transect” did not provide sufficient data to permit statistical analysis. The Chi-Square test was used to investigate differences between the number of females and males captured, this analysis being undertaken using STATISTICA 7.0 (StatSoft Inc.), with a significance level of 0.05 to reject the null hypothesis. At Bosco della Mesola, the sampling activities carried out did not result in the detection of any individual of *C. cerdo*.

### *Closed vs open occupancy models*

Single species and single season for closed and open occupancy models (MacKenzie et al. 2006, Kendall et al. 2013) were applied to test whether the population was open or closed. The following models were used: capture probability constant ( $\hat{p}$ , i.e. detection probability constant between surveys), full time ( $p$ , i.e. detection probability changes between surveys) or constrained time dependence ( $p_c$ , i.e. detection probability within the constrained time intervals is different from detection probabilities of time intervals before and after that constraint). Constant and full time dependence hypothesis refer to closed models. Instead, the constrained models take into account the entry probability ( $e$ , i.e. the emergence and/or colonisation of new individual) and the departure probability ( $d$ , i.e. the death and or emigration of individuals). For this analysis, the pooled data set, obtained from all traps during the entire study periods for both 2014 and 2016, was used.

### *Multi-method occupancy model*

In 2014, the effects of different baits in relation to the height of the traps above ground were tested. In 2016, based on the results obtained in 2014, only traps with the best bait and at the best height were set; additionally, a different ageing of the bait was tested. Single species, single season and multi-method occupancy models (Nichols et al. 2008) were applied to estimate the detection probability ( $\hat{p}$ ) for each sampling method ( $\cdot$ ). Detection probability was modelled as constant over time and baits or ageing ( $p$ ), as time-independent but different amongst baits or ageing ( $p_b$ ), as time-dependent but constant amongst baits or ageing ( $p_t$ ), or with baits or ageing as an additive effect with time-period ( $p_{s+t}$ ). Small-scale occupancy, which corresponds to the presence of the species at the local sample station, was modelled as either time-independent ( $\theta$ ) or time-dependent ( $\theta_t$ ). For these models, the data set of bottle traps with different baits at different heights for 2014 and the data set of traps with different states of ageing for 2016 were used. Both data sets refer to the time interval between the first and the last capture of each study period. The data set for 2014 refers to the time interval between

the first and the last capture of the study period whereas the data set for 2016 refers to the surveys from numbers 13 to 24. This period was selected to test the effect of the age of bait as, only from the 13<sup>th</sup> survey onwards, the baits A<sub>Nc</sub> and B<sub>3Ws</sub> had sufficiently aged to permit meaningful comparison.

### Covariates effects

Single species, single season occupancy models (MacKenzie et al. 2006, Kendall et al. 2013) were applied to test the magnitude of the effect of site covariates. In 2014, two site covariates were registered during the study: Tree Diameter (TD) measured at 1.3 m height, and Tree Species (TS), *Q. cerris* or *Q. robur*. In 2016, only one site covariate was registered: Number of Suitable Neighbourhoods (NSN). The magnitude of the effect of each survey covariate was modelled as constant ( $p_{\text{SURVEY COVARIATE}}$ ) or time dependent ( $p_{t\text{SURVEY COVARIATE}}$ ). The relationship between *C. cedo* occupancy ( $\psi$ ) and survey covariates was tested for the data from 2014 on first and second order polynomial models (TD, TS; TD+TD<sup>2</sup>, TS+TS<sup>2</sup>), hypothesising single, additive (TD+TS; TD+TD<sup>2</sup>+TS+TS<sup>2</sup>) and multiplicative effects (TD\*TS; TD+TD<sup>2</sup>\*TS+TS<sup>2</sup>). For the data of 2016, only first order polynomial models were considered (NSN). For these models, reduced data sets obtained from the best bait at the best height for 2014 and from the best state of ageing for 2016 were used. Both data sets refer to the time interval between the first and the last capture of *C. cedo* of the respective best method.

Models were ranked according to their values of AIC (Akaike Information Criterion), with models having low AIC value (i.e. more support) being ranked first (Burnham and Anderson 2002). Analyses were carried out using the programme PRESENCE (Hines and MacKenzie 2004).

To evaluate the survey effort necessary to achieve a standard error (SE) of 0.05 for the occupancy estimator  $\hat{\psi}$  given the calculated  $\psi$  and  $p$ , the value of  $s$  (number of sites to investigate, i.e. in this case the number of traps) and  $K$  (number of surveys) were evaluated according to the equation of MacKenzie and Royle (2005):

$$var(\hat{\psi}) = \frac{\psi}{s} \left[ (1 - \psi) + \frac{(1 - p^*)}{p^* - Kp(1 - p^*)^{K-1}} \right]$$

where  $p^* = 1 - (1 - p)^K$  is the probability of detecting the species at least once during  $K$  surveys of an occupied site.

The purpose of this analysis was to determine what values of  $s$  and  $K$  could be used to most efficiently achieve the desired level of precision for the value of occupancy ( $\hat{\psi}$ ) using the different trap types. The values of  $\hat{\psi}$  and  $p$  in the equation were the ones resulting from the best model previously selected.

## Results and Discussion

### Capture data

In 2014, VES did not provide any sightings whereas baited traps provided 29 captures of 28 individuals of *C. cerdo*, with no significant difference between female (16) and male (13) captures ( $\chi^2_1 = 0.31$ ,  $P > 0.05$ ); no beetle was found dead inside the traps. In 2015, the only method undertaken, Collecting of remains, provided three specimens. In 2016, VES provided the sightings of four individuals whereas Collecting of remains gave three specimens of *C. cerdo*. The baited traps provided 256 captures, with no significant difference between female (115) and male (141) captures ( $\chi^2_1 = 1.32$ ,  $P > 0.05$ ); five beetles were found dead inside the traps. Table 3 shows captures data performed by baited traps during the sampling season at Bosco della Fontana.

The phenology of the species for both years is shown in Figure 8. In 2014, the first capture was undertaken on 6 June (23<sup>rd</sup> week of the year), while the last capture was undertaken on 1 July (27<sup>th</sup> week of the year). In 2016, the first capture was undertaken on 3 June (22<sup>nd</sup> week of the year) while the last capture was undertaken on 22 July (29<sup>th</sup> week of the year).

### Occupancy models

#### *Closed vs open occupancy models*

For both years, the hypothesis of constrained time dependence for capture probability was strongly supported (Table 4, *Closed vs open models*). In both years, the highest

**Table 3.** Summary of captures data for *Cerambyx cerdo* recorded in 2014 and 2016 at Bosco della Fontana, using baited traps with different baits ( $R_w B_e B_a$  = red wine, beer, banana;  $R_w W_w S$  = red wine, white wine, sugar), different ageing (never = mixture never changed within comparison period; 2 weeks = mixture changed every 2 weeks, i.e. twice; 3 weeks = mixture changed every 3 weeks, i.e. once; over = mixture went over the comparison period), located at different heights (High = 10 m; Low = 1.5–2 m).

Year	Bait	Ageing	High	Low
2014	$R_w B_e B_a$	never	3	0
	$R_w W_w S$	never	21	5
	Control	–	0	0
2016	$R_w W_w S$	2 weeks	74	–
	$R_w W_w S$	3 weeks	50	–
	$R_w W_w S$	never	100	–
	$R_w W_w S$	over	32	–

**Table 4.** Summary of plausible models ( $\Delta\text{AIC} < 2$ ) obtained by model selection statistics for *Cerambyx cerdo*. Detection/non-detection data were recorded during the two years' study carried out at Bosco della Fontana in 2014 and 2016.

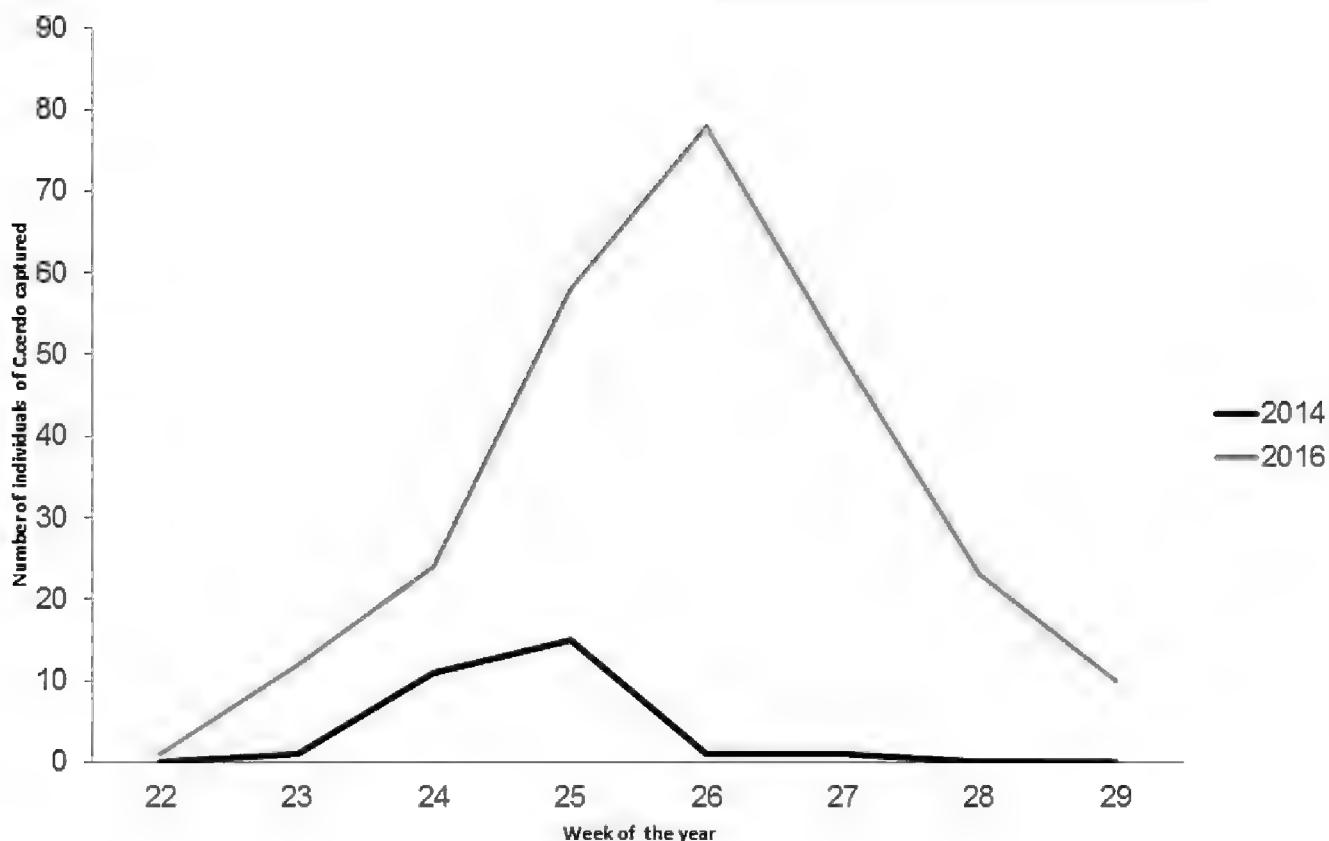
Year	Analysis	Model	K	-2Log(L)	$\Delta\text{AIC}$	w
2014	<i>Closed vs open models</i>	$\psi, e_c, d_c, p_c$	6	89.74	0.00	0.53
		$\psi, 0, p_s$	5	91.37	0.00**	0.98
	<i>Multi method Low</i>	$\psi, p$	2	51.31	0.00**	0.49
		$\psi, 0, p_s$	5	38.77	0.76**	0.33
	<i>Covariates effects</i>	$\psi_{\text{TS}^*\text{TD}}, p$	2	65.09	0.00	0.32
		$\psi, p$	3	63.73	0.64	0.23
		$\psi_{\text{TD}}, p$	3	64.33	1.24	0.17
		$\psi_{\text{TS}^*\text{TD}}, p$	4	62.68	1.59	0.14
		$\psi_{\text{TS}}, p$	3	64.80	1.71	0.13
2016	<i>Closed vs open models</i>	$\psi, e_c, d_c, p_c$	6	574.35	0.00	0.60
		$\psi, 0, p_s$	5	603.68	0.00	0.50
	<i>Multi method</i>	$\psi, 0, p_{s+t}$	16	581.94	0.26	0.44
	<i>Covariate effect</i>	$\psi, p_t$	13	209.96	0.00	0.8046

Large scale occupancy ( $\psi$ ), small scale occupancy ( $\theta$ ), detection ( $p$ ), entry ( $e$ ) and departure ( $d$ ) probabilities are considered. Constraints:  $c$  = constrained time dependence (hypothesis where  $p$  between the first and last capture is different from the  $p$  observed during the other weeks);  $s$  = methods, i.e. baits or ageing;  $t$  = full time dependence. Site covariates: TD = tree diameter; TS = Tree species; \* = interactive effect; + = additive effect. Survey covariates: RH = relative humidity; TE = temperature. COVARIATE+COVARIATE<sup>2</sup> = second order polynomial relationship. K represents the number of parameters in the model; w the Akaike weight and -2Log (L) is twice the negative log-likelihood value. Akaike Information Criteria ( $\Delta\text{AIC}$ ) were calculated for each model. The \*\* indicate the use of relative Quasi-Akaike Information Criteria ( $\Delta\text{QAIC}$ ), i.e. the AIC modified for overdispersion (Burnham and Anderson 2002).

conditional entry probabilities ( $e$ ) were found within the constrained sampling period, whereas the highest conditional departure probability ( $d$ ) were found outside of the constrained sampling period (Table 5).

### **Multi method occupancy models**

In 2014, bait (R<sub>w</sub> W<sub>w</sub> S) and height of the traps strongly influenced the detection probabilities of *C. cerdo* (Table 4, *Multi method High* and *Multi method Low*). Estimated detection probabilities were reported in Table 6. In 2016, the ageing of the bait strongly influenced the detection probability (Table 4, *Multi method*). The “never-changed” baits resulted in the highest value of detection probability: A<sub>N</sub> 0.26 ± 0.03, B<sub>3W<sub>s</sub></sub> 0.16 ± 0.03, C<sub>2W<sub>s</sub></sub> 0.16 ± 0.03 (values obtained from the top model  $\psi, 0, p_s$  of Table 4, Year 2016, *Multi method*).



**Figure 8.** Phenology of *Cerambyx cerdo* during the sampling season of 2014 and 2016 at Bosco della Fontana.

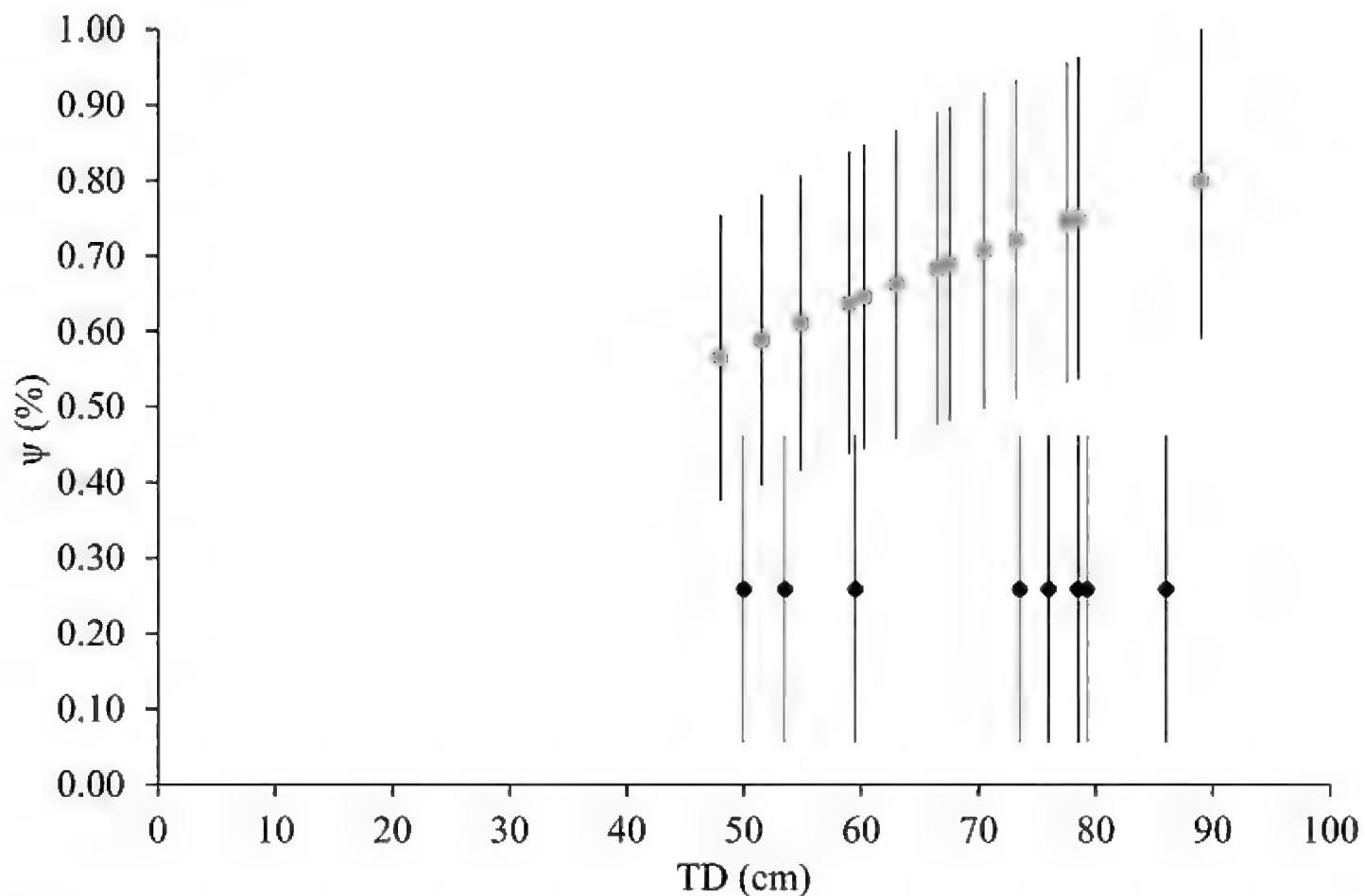
**Table 5.** *Cerambyx cerdo* entry ( $e$ ) and departure ( $d$ ) probability estimates and associated standard errors (SE) are given for the top models.  $w$  is the Akaike's weight for each model. Detection/non-detection data were recorded during surveys carried out in 2014 and 2016.

Year	Model	w	Constrained time period (survey)	e (SE)	d (SE)
2014	$\psi, e_c, d_c, p_c$	0.53	1–4	0.00 (0.00)	1.00 (0.00)
			5–26	0.32 (0.13)	0.15 (0.07)
			27–31	0.00 (0.00)	1.00 (0.00)
2016	$\psi, e_c, d_c, p_c$	0.6	1–3	0.00 (0.00)	1.00 (0.00)
			4–32	0.14 (0.03)	0.03 (0.01)

**Table 6.** *Cerambyx cerdo* detection probability estimates ( $\hat{p}$ ) and associated standard errors (SE) are given for the top models obtained for bottle traps (Con = control;  $R_w B_e B_a$  = red wine, beer, banana;  $R_w W_w S$  = red wine, white wine, sugar) at different heights (High = 10–20 m; Low = 1.5–2 m).  $w$  is the Akaike's weight for each model. Detection/non-detection data were recorded during surveys of 48 bottle traps carried out at Bosco della Fontana between May and July 2014.

Bottle traps	Model	W	$\hat{p}_{Con}$ (SE)	$\hat{p}_{RwBeBa}$ (SE)	$\hat{p}_{RwWwS}$ (SE)
High	$\psi, \theta, p_c$	0.98	0.00 (0.00)	0.05 (0.03)	0.31 (0.09)
Low	$\psi, \theta, p_c$	0.49	0.03 (0.03)	0.03 (0.03)	0.03 (0.03)

\* Parameter estimates reported are the mean values amongst the detection probability estimates for each daily check.



**Figure 9.** Estimated occupancy probability ( $\psi$ ) in relation to tree species (dark grey = *Quercus cerris*; light grey = *Quercus robur*) and tree diameter (TD). Vertical bars show the standard errors (SE). Detection/non-detection data were recorded during surveys of baited bottle traps located in the Bosco della Fontana from May to July 2014.

### Covariates effects

When *C. cerdo* was sampled in 2014 by baited traps with the best method ( $R_w W_w S$ ) at the best position (High), between the first and last capture (Surveys 8-16), closed models were supported. For *C. cerdo*, statistical modelling reflects selection model uncertainty on species occupancy (Table 4, *Covariates effects*). Estimates of the relative importance for site covariates suggest an interactive effect between TS and TD ( $w_{TS*TD} = 0.32$ ) (Table 4, *Covariates effects*; Figure 9). The occupancy of *C. cerdo*, tested in 2016, was not affected by NSN (Table 4, *Covariates effects*). To achieve a SE of 0.05 for the estimate of the parameter  $\psi$ , using the values obtained for  $p$ , with the traps set high, baited with  $R_w W_w S$  and in which the mixture was not changed during the season, it is sufficient to use 10 traps which are checked 15 times during the experimental period. In other words, 10 traps checked 15 times during the experimental period are sufficient to detect the species with a high degree of certainty (SE 0.05) if the species occurs in the area.

## The standard method for the monitoring of *Cerambyx cerdo*

### *Description of the proposed monitoring method*

From the results obtained, the use of baited traps was proposed with some practical considerations as the standard method for the monitoring of *C. cerdo*. Traps baited with red wine, white wine and sugar, positioned over 10 m high resulted in the best combination to detect the target species at Bosco della Fontana. The most important factors correlated with the capture of *C. cerdo* are the diameter of the tree (capture probability increasing with the increase in the DBH) and the tree species (*Q. robur*). At Bosco della Fontana, *Q. robur*, *Q. cerris* and *C. betulus* represent the co-dominant tree species, thus the choice of the tree for positioning of the trap should be undertaken by considering the preferences of *C. cerdo* for oak species and the native hardwood species occurring in the study area (Weckwerth 1954, Döhring 1955, Neuman 1985).

The choice of the tree species should be guided by the following considerations: (i) Searching for oak with greater DBH available (at least 50 cm if possible), (ii) Searching for oaks, living or partially dead with damage at the trunk or branches and a suffering but still vital canopy and (iii) Searching for colonised trees, with visible holes characterised by wood meal and red-coloured interior.

A partially dead tree, currently suitable for monitoring, will not be suitable after some years when degradation of the wood will progress and the tree will eventually die. Thus, for any long term monitoring programme, it is clear that the single tree initially selected will have to be replaced by other trees which will become suitable in future years. Any choice of trees to be surveyed should consider the long term monitoring of an area and hence the changes which the trees will face in the future in order to plan forest management and protect biodiversity in case of mandatory cuts. The statistical analysis of these data showed that, although the occupancy of *C. cerdo* was not affected by the number of suitable trees in the neighbourhoods, this could be influenced by the homogeneity of the forest at Bosco della Fontana, characterised by many oaks suitable for *C. cerdo*. Thus, in a forest characterised by a greater heterogeneity in terms of tree composition, the presence of large trees (DBH  $\geq$  50 cm) and partially dead trees, which are suitable for monitoring, it is probably advisable to add these trees to those selected for the standard monitoring programme.

As explained in sampling plan, the trap consists of two jars: the lower one containing the bait as liquid mixture, the upper one as the capture chamber. The two jars are separated by a wire net to ensure the survival of the specimens in the trap and avoiding any contact with the liquid. In practice, each litre of mixture was formed by 50% of red wine and 50% of white wine (500 cm<sup>3</sup> for each) with the addition of 220 g of sugar. The mixture should be prepared a week before the trap setting in order to obtain a bait with an initial degree of fermentation and to allow the sugar to dissolve completely in the mixture. As demonstrated, the bait, whose mixture was

never changed, resulted in the highest value of detection probability, compared to the mixtures replaced every three and two weeks respectively. Thus, during the entire sampling period, the mixture should never be changed, except for topping up when the mixture falls below the level of 500 cm<sup>3</sup> due to evaporation. It is recommended to carefully attend to the traps exposed to sun, indicate the correct level of the liquid with a marking pen on the jar and to quickly check the amount of the mixture during each daily control. Traps should be positioned in each study area, at least for the first time, at two height positions: on the trunk at about 1.5–2 m high and on branches over 10 m. According to the sampling plan, in 2014, traps were positioned at two heights above ground to evaluate the presence of the species both at the underground level and canopy lower level. After the extremely low detection probability of *C. cerdo* in the underground level of Bosco della Fontana was found, only the higher traps were set in 2016. The low number of *C. cerdo* sightings at the underground level of Bosco della Fontana could be due to the highly shaded condition of the understory. This hypothesis could be corroborated by the very low number of trees with exit holes at the base of the trunk detected in this study area, rather than on the highest branches which had fallen on the ground. (Author's personal observation).

The locations of each pair of traps (low and high) should be chosen to facilitate their setting, mostly the higher ones and also to make them easy to check. It is therefore recommended to set the traps on suitable trees along forest roads avoiding steep terrain. As explained in the sampling plan section, for a baited trap positioned at a lower canopy level, a tree-climb slingshot (BigShot by Sherrill tree) is used for the launch of the rope, to which the trap is then tied. During the launch of the rope, there should be enough space around. Thus in a dense forest, the only suitable trees could be located along the forest road. The standard monitoring protocol (Table 7) needs to be repeated at the same site in future years without any changes in methodology, to ensure that the data gathered provide reliable information on eventual changes in local population abundance and can be compared chronologically as well as with other areas investigated. In each area, 10 trees should be selected; a distance of at least 100 m between them is suggested. A pair of baited traps (one low and one high) per tree must be set for monitoring. In case of very small woodlands with few suitable trees ( $n < 10$ ), the number of paired traps and/or the distance between the trees should be reduced. Traps should be checked daily, three times a week for five weeks. It is recommended to check the traps in the morning (from 08:00h to 11:00h). Traps should be activated on Monday and remain active until Thursday being checked in three consecutive mornings. After the last check, traps must be temporarily deactivated by closing the jar with a lid and removing the one modified with the funnel. The duration of each survey (checking ten traps, high and low) depends on the number of captured individuals and on the distribution of the traps in the study area; as an indication, two hours should be sufficient for each survey.

Our results demonstrate that the application of the proposed protocol in terms of number of traps, frequency checks and the number of monitoring weeks allows a SE of 0.05. Furthermore, our results suggest that starting should be at the 23<sup>rd</sup> and ending

**Table 7.** Summary of the monitoring protocol for *Cerambyx cerdo*.

Monitoring protocol	
Method	Baited trap
Number of trees	10
Number of baited traps	20 traps for each site
Position on tree	One trap on the trunk (1.5–2 m high); the other on branches (over 10 m high)
Placement of baited traps	On trees along forest roads or pathways
Distance between trees with baited trap	At least 100 m
Monitoring period	June–July
Number of weeks	5
Number of surveys	15
Frequency of surveys	Three a week
Time of the day	08:00–11:00h
Number of operators	2
Hours per person	40
Equipment	A clipboard, a field sheet, a pencil, GPS, a rope, two replacement jars, bottles with mixture

at the 27<sup>th</sup> week of the year (June and early July) but this period should be adjusted according to previous knowledge about the population phenology of *C. cerdo* observed at local level. If the local phenology of the species is unknown (or cannot be reasonably inferred from available data), exclusively for the first year of monitoring, it is recommended to begin the sampling earlier from the 21<sup>st</sup> week of the year.

The standard method, described here, is based exclusively on counts of *C. cerdo* individuals captured. If additional aspects of the local population are to be investigated (e.g. population size, life expectancy etc.), the monitoring protocol proposed can be extended using the capture-recapture protocol. During MIPP fieldwork, this technique was successfully applied using tags for queen bees (<http://www.enolapi.net/wordpress/prodotti/bollini-segnare-le-regine/>) glued to the elytra of the adults by Loc-tite Super Attack Power Flex Gel) (Figure 10).

## Protocol, materials and equipment

The first step involves the selection of the tree on which the baited trap will be set up, after identifying a suitable tree according to the characteristics explained above i.e. DBH ≥ 50 cm, living or partially dead and with signs of the presence of *C. cerdo*; the selected tree must be identifiable by a unique numerical code and its geographical position registered with a GPS in order to locate each single tree. During the selection of the trees, it is important to set the ropes in place on branches over 10 m for the higher traps using the sling shot, as described in the sampling plan.



**Figure 10.** An individual of *Cerambyx cerdo* captured with a baited trap and bearing the glued tag (Photo by M. Bardiani).

The second step involves the preparation of the bait. The mixture should be prepared a week before the trap setting in order to obtain a bait with an initial degree of fermentation and to allow the sugar to dissolve completely in the mixture. Each litre of mixture is formed by 50% of red wine and 50% of white wine (500 cm<sup>3</sup> of each), with the addition of 220 g of sugar. On the day established for starting the sampling activity, the mixture should be distributed inside the lower jar of each baited trap.

The third step involves the setting up of the ten baited traps: on each tree, one baited trap low and one baited trap high should be set. The lower trap must be positioned at 1.5–2 m on the trunk. Once the traps are positioned, the lid of the upper jar should be removed and replaced by the lid modified with the funnel.

The fourth and last step involves the checking of the traps. The traps should be checked three times a week, during the period of maximum activity of *C. cerdo*, when weather conditions are favourable; if weather conditions are not favourable on a pre-selected day, it is advisable to carry out the fieldwork on another day as soon as possible to prevent the death of the beetle inside the trap. Once the checking of the traps has been completed, the number of individuals collected should be counted, specifying the number of males and females. After the compilation of the fieldsheet

(See Suppl. material 2: Field sheet 2), the individuals must be released on to trees around the traps.

### ***Spatial validity, constraints and possible interferences***

In the present study, the capture-recapture protocol applied in 2016, has been used to calculate the distances covered by recaptured individuals. This calculation showed that adults of *C. cerdo* can move on average  $750\text{ m} \pm 309\text{ m}$  standard deviation (SD). Therefore, it is assumed that the validity of the results of the monitoring extends to an area surrounding the tree selected for baited traps to a maximum of 1000 m. If the average distance between the 10 selected trees investigated is 100 m and if one calculates the area which extends to a maximum of 1000 m from these trees, an area of about 300 ha is obtained. This area represents the forest surface for which the results of the monitoring are assumed to be valid. If the monitored area is located within a homogeneous forest (for tree composition, tree age, tree management, dead wood amount etc.), the validity extends to the whole of this area.

The major constraints of this method involve the obligatory daily check of the traps in order to avoid any injury or death of the individuals collected and dangerous diurnal temperatures inside the plastic traps during the day.

A possible interference on the use of baited traps is related to the presence of the dormouse (*Glis glis*). In fact, as reported by Bardiani et al. (2017a) this rodent could inhabit the trap, preventing the collection of the beetles or killing the individuals captured.

In areas which are accessible by people, the trap set low on trunks and the points where the ropes of the high trap are tied (e.g. small shrub branches), are easily visible and approachable by visitors. For the tied points of ropes, it is suggested that they be hidden as far as possible but in general, the use of explanatory signs about the monitoring and the function of the trap might be the best way to inform people and to try to avoid any possible interferences.

In addition, the use of baited traps could influence the monitoring of other beetles (e.g. stag beetles, flower chafers) which are attracted by this kind of bait (Bardiani et al. 2017b).

### ***Counting, quantification and data sharing***

In order to assess the conservation status of populations of *C. cerdo* for a given season and for a given area, a reference value is calculated as follows (Table 8):

1) For each week, calculate the total number of individuals (males + females) by adding up the number of individuals found in each baited trap. It is recommended to separately report the number of individuals captured by low and high traps.

2) Calculate the mean values of individuals captured in each week and for each type of trap (H and L).

**Table 8.** An example of calculation of the total and mean value of the individuals counted. The mean value obtained is the reference number to compare the long-term data and to identify a population trend. The range of values obtained during the MIPP project varied between 29 captures with 8 baited traps (Bosco Fontana 2014) and 256 captures with 54 baited traps (Bosco Fontana 2016). (BT = baited trap, H= high, L=low)

	BT1		BT2		BT3		BT4		BT5		BT6		BT7		BT8		BT9		BT10		Total per week	Mean value per week
	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L		
Week1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	3	0.15
Week 2	1	1	1	1	0	0	2	0	1	0	2	0	0	1	0	0	1	0	1	0	12	0.6
Week 3	1	3	0	1	1	0	1	1	4	1	5	4	3	1	4	1	3	0	4	0	38	1.9
Week 4	2	0	3	1	0	0	3	0	1	2	2	1	0	0	2	2	1	0	1	1	22	1.1
Week 5	1	1	0	1	1	0	0	0	2	0	0	0	1	0	1	0	0	0	0	0	8	0.4
Total per H/L	5	5	5	4	2	0	6	2	8	3	10	5	4	2	7	3	5	0	6	1	83	4.15
Mean number of captures per trap and week																						0.83

## Acknowledgements

Authors are grateful to J. Buse and E. Micó for their valuable comments on an earlier version of the manuscript. We would like to thank all colleagues, students and friends who gave a help during the surveys: E. Bussola, F. Delle Pezze, E. Mezzadri, B. Sall, D. Sogliani, L. Spada, I. Toni and M. Vega. The authors are grateful to the local offices of the Comando Unità Tutela Forestale Ambientale ed Agroalimentare Carabinieri which manage the study sites: the UTCB Punta Marina (Dr. G. Nobili, M. Menghini, P. Benini) and the UTCB Bosco Fontana (Dr. V. Andriani, F. Mazzocchi and L. Fedrigoli). We would like to thank M. Tisato and M. Lopresti who provided assistance for the maps of the study areas. We are also grateful to all field assistants who voluntarily helped us during the surveys: M. Boscaro, F. Grant, S.G. Muñoz, J. Röeder, G. Rutten, R. Santoro, A. Vannini and G. Zanettin. We would like to thank P. Rapuzzi, for allowing us to use his photos and for technical advice on longhorn ecology and behaviour. The present work was developed within the EU project LIFE11 NAT/IT/000252, with the contribution of the LIFE financial instrument of the European Union.

A special permit was obtained from the Italian Ministry of Environment for handling and capturing individuals of the target species (collection permit: Ministero dell'Ambiente e della Tutela del Territorio e del Mare - DG Protezione della Natura e del Mare, U.prot PNM 2012-0010890 del 28/05/2012).



With the contribution of the LIFE financial instrument of the European Union.

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## Supplementary material I

### Field sheet 1

Authors: Lara Redolfi De Zan, Marco Bardiani, Gloria Antonini, Alessandro Campanaro, Stefano Chiari, Emiliano Mancini, Michela Maura, Simone Sabatelli, Emanuela Solano, Agnese Zauli, Giuseppino Sabbatini Peverieri, Pio Federico Roversi

Data type: field sheet

Explanation note: Field sheet for choosing the most suitable trees for the baited traps.

The operator should mark with an “x” the corresponding box for the status of the canopy and the bark, the presence or not of sap or exit holes. It is also useful to write the presence of suitable trees around those selected.

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Link: <https://doi.org/10.3897/natureconservation.20.12703.suppl1>

## **Supplementary material 2**

### **Field sheet 2**

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Data type: field sheet

Explanation note: Field sheet to be compiled during each survey (three a week for five weeks, 15 on the whole). For each trap checked, the operator must write the number of individuals captured, divided by sex and for trap height.

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